

## IMMUNE CELLS INFILTRATION IN ADIPOSE TISSUE OF GREATER OMENTUM

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**Background.** Dickinson GK has developed several hypotheses on immune function of greater omentum in article published in 1906 in *Annals of Surgery*, and this article likely was the first scientific publication for greater omentum function. Particularly he suggested that greater omentum: (1) through its readiness to lymph formation and local proliferation, it becomes attached to infected parts, which are walled off, subsequently to be absorbed by phagocytic action; (2) The majority of the phagocytes extruded into the peritoneum for its protection come through the omentum, largely from the general circulation, but in part from the tissues therein existing; subsequently to be attached to the surface of this tissue, taken into the lymph-stream, and subjected to the cytolytic influences existing there.<sup>1</sup>

Nova days general principles of these concepts are not changed but enriched, and therefore recognized greater omentum's main role in protection of abdominal cavity, its ability in adhesion and elimination of microbial and tissue damage products and its support for tissue regeneration through angiogenesis activity.<sup>2, 3</sup> Embedded within the omentum are opaque structures, which are clusters of leukocytes, called milky spots. Milky spots are mainly composed of macrophages and B1 cells, resembling the cellular composition found in the peritoneal cavity.<sup>4</sup> There were reported that omentum collected antigens and cells from the peritoneal cavity and supported T cell-dependent B cell responses, including isotype switching, somatic hypermutation, and limited affinity maturation, despite the lack of identifiable follicular dendritic cells.<sup>5</sup>

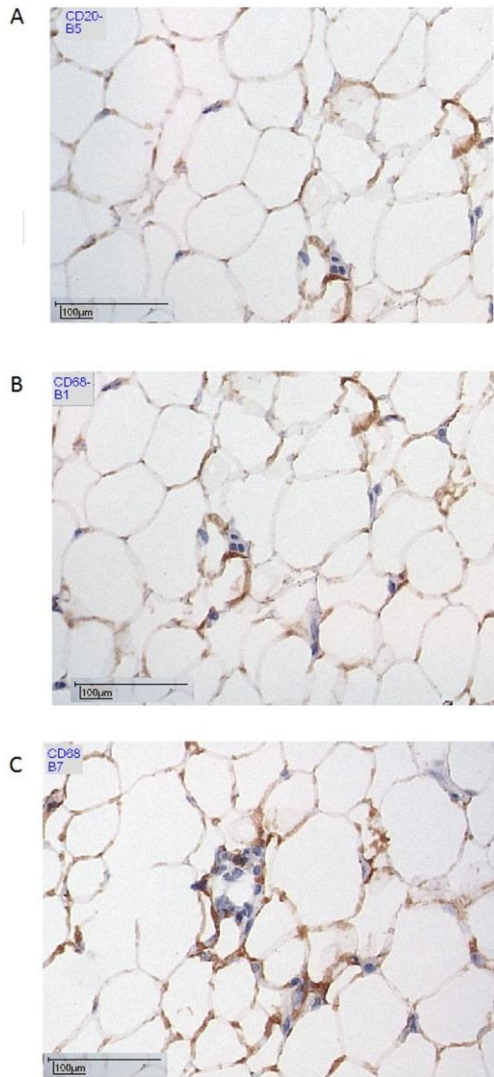
In this study we have aimed to determine composition and level of infiltrated greater omentum immune cells in patients with abdominal trauma during the emergency surgical intervention.

### Materials and methods.

Study was provided from October 2013 to June 2014 in Department of Pathology and Pathophysiology and Department of Microbiology and Immunology, School of Pharmacy and Biomedicine, Mongolian National University of Medical Sciences, in Clinics of Emergency of in United Hospital of Defense Servers and Guardians of Justice and National Institute of Forensic Medicine, Ministry of Justice, Ulaanbaatar city, Mongolia.

*Design.* Patient based descriptive study design was used for the study.

*Specimen.* There were investigated resected parts of greater omentum obtained from 39 patients (28 males, 11 females) operated regarding with closed or open trauma of abdominal cavity in United Hospital of Defense Servers and Guardians of Justice. Greater omentum autopsy materials for comparison were obtained from 22 bodies of people died suddenly after accident and investigated at National Institute of Forensic Medicine.



**Figure 1 – Immune cell infiltration rate in greater omentum**

Notes: A – surgical material, 36 years old male patient, IHC, anti-CD20,  $\times 400$ , own observation; B – surgical material, 29 years old female patient, IHC, anti-CD68,  $\times 400$ , own observation; C – surgical material, 44 years old male patient, IHC, anti-CD20,  $\times 400$ , own observation;

*Patients.* Mean age of patients who undergone the abdominal surgery was  $38.9 \pm 12.6$  (22–57) year. In 16 (41%) of patients have been diagnosed closed trauma of abdominal cavity and in rest 23 (59%) were found perforating wound of abdominal cavity. Average time from accident to surgical intervention was  $9.82 \pm 4.76$  (4–23) hours. All patients were left the hospital within  $11.2 \pm 4.8$  (8–21) days.

20 (90.9%) of sudden death cases was caused by traffic accident, one of rest two cases was pressed by earth movement with earth and another case was caused by fall from roof. Average time from death to obtain of autopsy was  $14.7 \pm 3.8$  (9–19) hours.

*Immunohistochemistry (IHC).* Greater omentum specimens were processed according with standard methods of paraffin embedded tissue processing. Tissue slides were prepared and stained with monoclonal antibodies to CD4, CD8, CD20, CD25, CD57 and CD68 antigens using immunohistochemistry staining kit (MaiXin, China).

*Evaluation of immune cell infiltration.* Slides was examined by light microscopy. Structures of greater omentum resembling milky spot were examined under  $\times 400$  magnifications. Number of stained cells by given antibody to each given antigen was counted in 5 fields of vision (Count of immune cells). Average number of stained cells and its standard deviation ( $M \pm SD$ ) was calculated. If SD is greater than 15% of M another 5 fields of vision was examined. Infiltration rate of immune cells was evaluated by scores (1–3)<sup>6</sup>: 0 – no infiltration or no stained cells; 1 – weakly infiltrated or mean of stained cells is between 1–5 (figure 1A); 2 – moderately infiltrated or mean count of stained cells is between 6–20 (figure 1B); and 3 – strongly

infiltrated or mean count of stained cells is more than 20 (figure 1C).

Significance of difference in immune cells infiltration rate in different clinical groups (type of trauma, target damaged organ, time after exposure and contamination state) was calculated using Pearson's coefficient ( $\chi^2$ ).

*Ethical considerations.* Methods of this study were reviewed in the meeting of the Institutional Review Board of Mongolian National University of Medical Sciences and concluded that the statement in the methods complied with the requirements of experiments on human subjects and were issued a resolution No. 16 dated 04 October 2013.

### Results

General profiles of 61 cases enrolled in the study are shown in the table 1.

Table 1 – General profiles of cases enrolled in the study

	Gone under surgery (n=39)	Sudden death (n=22)
Mean age(M±SD)	41.0±15.6	47.6±16.9
Age groups (n/%)		
<30	11/28.2	5/22.7
31–40	13/33.3	3/13.6
41–50	4/10.3	3/13.6
51–60	5/12.8	7/31.8
61<	6/15.4	4/18.2
Sex (n/%):		
Males	28/71.8	20/90.9
Females	11/28.2	2/9.1
Trauma type (n/%)		
Closed abdominal trauma	16/41.0	
Break of hollow organs	10/62.5	
Break of parenchymal organ	6/37.5	
Perforated wound	23/59.0	
Perforated trauma of hollow organ	11/47.8	
Perforated trauma of parenchymal organ	1/4.4	
No organ trauma	11/47.8	
Contamination state (n/%):		
Visible contamination	13/33.3	
No visible contamination	26/66.6	
Time until operation(hour, M±SD)	9.82±4.76	
Subgroups (n/%)		
<6 h	15/38.5	
7–12 h	12/30.8	
13–18 h	9/23.1	
19–24 h	3/7.6	
Cause of death (n/%)		
Traffic accident		20/90.9
Pressed by earth movement		1/4.5
Fall		1/4.5
Time until autopsy(h, M±SD)		14.7±3.8
Subgroups (n/%)		
Until 12 h		7/31.8
Later 12 h		15/68.2

In Table 2 shown frequency of immune cells infiltration rate in greater omentum of patients undergone the emergency surgical operation in comparison with that of suddenly dead subjects.

Table 2 – Immune cells infiltration rate frequency

Stained antigens		Scores of infiltration rate <sup>a</sup>							
		0		1		2		3	
		n	%	n	%	n	%	n	%
CD4+	SO <sup>b</sup>	2	5.1	18	46.2	14	35.9	5	12.8
	SD <sup>c</sup>	19	86.4	3	13.6	–	–	–	–
CD8+	SO	15	38.5	20	51.3	4	10.3	–	–
	SD	20	90.9	2	9.1	–	–	–	–
CD25+	SO	17	43.6	17	43.6	4	10.3	–	–
	SD	22	100	–	–	–	–	–	–
CD20+	SO	2	5.1	24	61.5	12	30.8	1	2.6
	SD	19	86.4	3	13.6	–	–	–	–
CD57+	SO	16	41.0	17	43.6	6	15.4	–	–
	SD	21	95.5	1	4.5	–	–	–	–
CD68+	SO	–	–	–	–	20	51.3	19	48.7
	SD	2	9.1	15	68.2	5	22.7	–	–

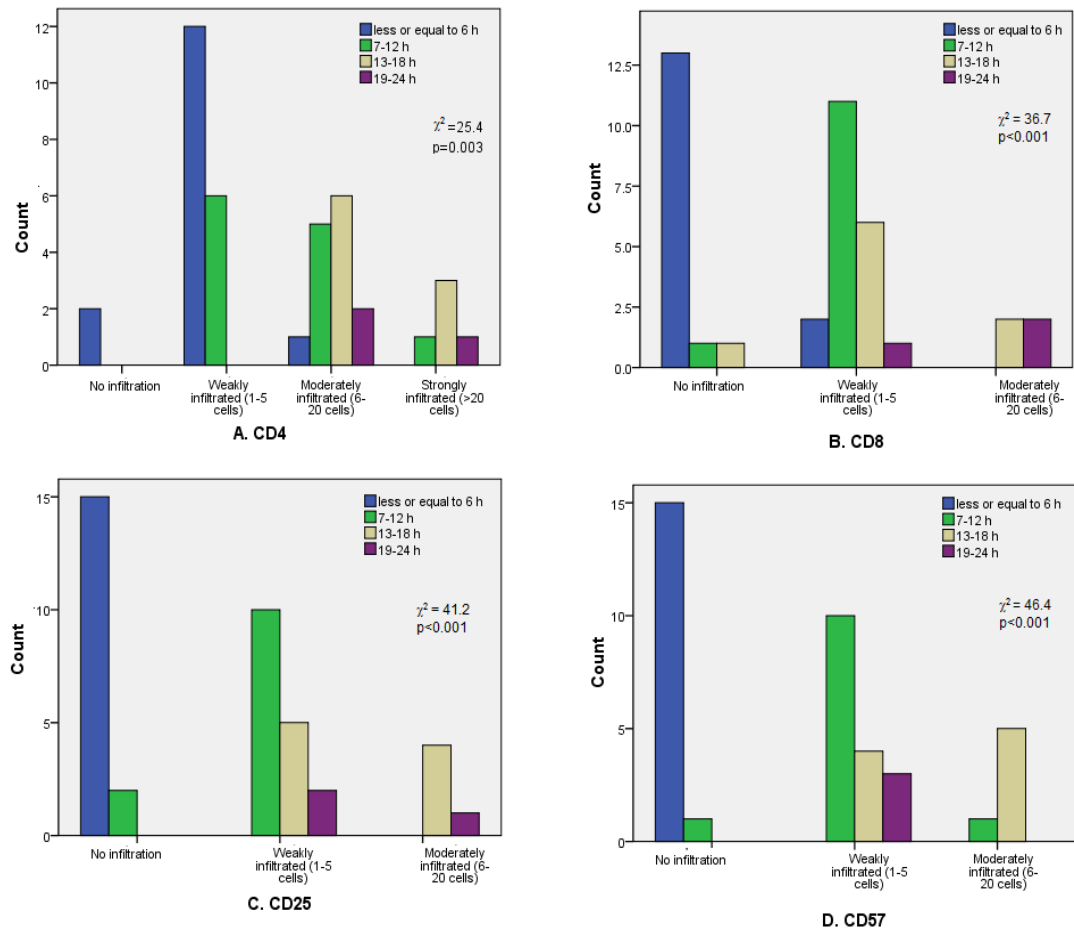
<sup>a</sup>–0 – no infiltration; 1 – weak infiltration(1–5cells); 2 – moderate infiltration(6–20cells); 3 – strong infiltration(>20cells). <sup>b</sup>–Surgical operation; <sup>c</sup>–Suddenly dead

As shown in table 2 more portion of cases with higher rate of immune cells infiltration were observed in group of operated patients compared with group of suddenly deceased subjects.

When compared immune cells infiltration rate in different groups of patients it was found no significant difference between patient age, sex and type of organ involved ( $p>0.05$ ). But time taken from accident to surgical operation has shown dramatic increase in infiltration rate of CD4+ (T helper cells), CD8+ (cytotoxic T cells), CD25+ (T regulatory cells) and CD57+ (natural killer) cells. (Figure 2).

There were not found significant increase of number of patients with higher rate of infiltration in CD20+ (B lymphocyte) and CD68+ (macrophage) cells.

More number of cases with higher rate of CD57+cell infiltration was demonstrated in group of patients with perforated wound of abdominal cavity in comparison with group of patients with closed abdominal trauma (Figure 3A), and elevated number of patients with higher rate of CD68+cell infiltration was observed in group of patients with visible contamination of abdominal cavity resulted perforating trauma of hollow organs (gut, bladder and gallbladder) compared with patients without visible contamination (Figure 3B).

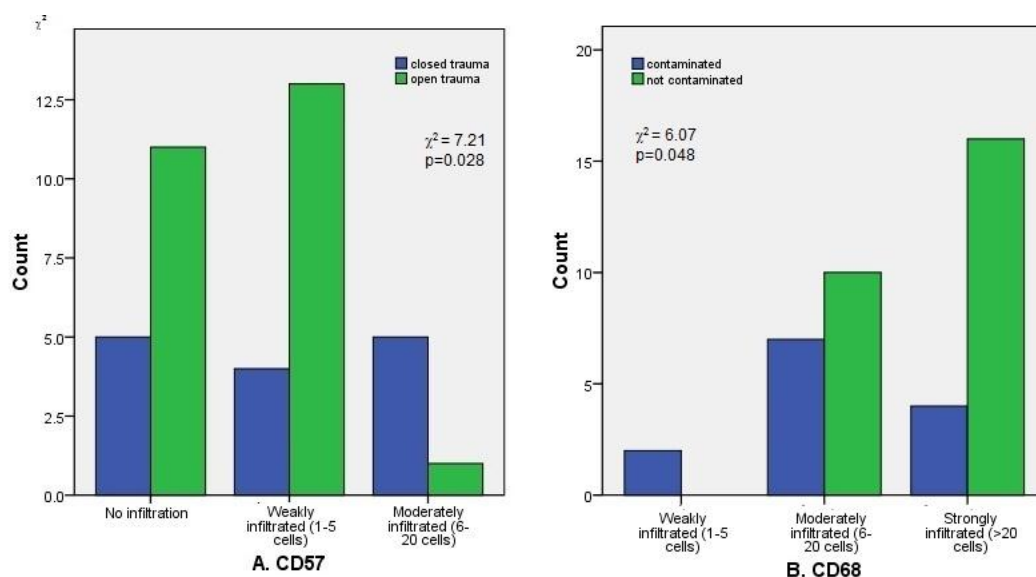


**Figure 2 –Time taken from accident to surgical operation and immune cells infiltration rate**

### Discussion.

There were reported that majority of immune cells of in milky spot of human greater omentum are presented with macrophages (70%), B lymphocytes(10%)and T lymphocytes(10%).<sup>7</sup>Krist LE etc.(1995)<sup>8</sup>analyzed immune cell composition in greater omentum of patients operated after pathologies not affecting milky spot state and found macrophagesin 67.9±9.4%, B lymphocytes in10.1±3.4% and T lymphocytes and mast cells 10.2±3.4% of cells.

We could not do direct comparison of our results with these findings because of there were counted separated from greater omentum tissue cells. We found increased average portion of moderately or strongly infiltration of all antigen bearing cells (CD4+, CD8+, CD20+, CD25+8 CD57+ and CD68+) in biopsy materials, but it was found in 90.9%,82.2% and 61.6% in greater omentum tissue of sudden death cases weak or moderate infiltration of macrophages (CD68+ cells), T helper cells (CD4+) and cytotoxic T cells (CD8+), respectively.



**Figure 3 – Immune cell infiltration rate in comparison with trauma type and contamination state**

Shimotsuma M., et al. (1992)<sup>9</sup> has observed changes in the surface features of milky spots and milky spot macrophages using scanning electronic microscopy and counted macrophage population in peritoneal exudates after different time points of intraperitoneal administration of streptococcal antigen OK-432 to Wistar rats. They found the number of milky spots increased between 5 and 12 inches each trimmed specimen following the injection of OK-432. Scanning electron microscopy showed that as early as 3 hour following the injection of OK-432, the number of macrophages increased on the surface of the milky spots, and by day 1 after the injection of OK-432, the milky spot was almost completely covered by macrophages. Although in the control group it was very rare to find a macrophage appearing through the stornata of a milky spot, in the experimental groups, especially at 12 hour of first day, and 3 days after the injection of OK-432, at least two or three macrophages were found to be appearing through the stornata of a milky spot. The macrophage population in 300 peritoneal exudates cells increased from 3 hour (8.4%) after the injection of OK-432, reached its peak on 3 days (42.7%), and also continued to 7 days following the injection (34.2%). In this study we can see very early migration of macrophages into adipose tissue of the omentum and it can explain why count of macrophages did not demonstrate significant difference in relatively late (9.82±4.76 hour) period from exposure in our observation.

Lymphocyte homing Carlow DA., et al. (2009)<sup>10</sup> concluded that the omentum incorporates two key features of immunological sentinel function, actively supported lymphocyte traffic and dendritic cells, that reinforce a conceptual framework for function in stimulating adaptive immunity. Berberich S., et al (2008)<sup>11</sup> demonstrated that  $\alpha_4\beta_7$  integrin-mucosal addressin cell adhesion molecule 1 interaction enables B2 cell migration from the circulation into omental milky spots but not into the peritoneum. Therefore they suggested that main sources for B cells in omentum are the peritoneal cavity. In our cases we could not demonstrate progressive migration of CD20+ B cells to omentum. It may be explained by dominant colonization of omentum adipose tissue by B1 lymphocytes, but not CD20+ B cells.

These findings have indicated relatively late, after support of antigen presenting cells homing of immunocompetent cells into milky spots.

In this study we observed intensive infiltration of immune cells in greater omentum of patients operated according with abdominal trauma with earlier than CD4+ and CD8+ T cells colonization of macrophages after traumatic exposure of abdominal cavity. These findings may be used as basics for selection of therapeutic approaches in period after surgical intervention in abdominal cavity.

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